**Case 11: Graft versus Host Disease (GVHD)**

**Summary:** Bone marrow transplants are used as treatment for leukemia, bone marrow failure (aplastic anemia), and primary immunodeficiency diseases. Other sources of hematopoietic stem cells include peripheral blood steam cells and cord blood can also be used as therapy. Bone marrow and other sources contain mature T cells, which may recognize the tissues of their new host as foreign and cause a severe inflammatory disease in the recipient known as graft vs. host disease. The immune system of the recipient must be destroyed and the recipient rendered immunoincompetent. This is accomplished by lethal doses of radiation or injection of radiomimetic drugs such as busulfan, and the use of immunosuppressive drugs, such as cyclophosphatmide and fludarabine. In kids with SCID who can’t produce T cells, this preparative treatment isn’t needed.

GVHD occurs when there is a mismatch of MHC I and II molecules, but also in the context of disparities in minor histocompatibility antigens. Minor differences such as these are likely to pbe present in all donor-recipient pairs other than identical twins even when HLA-matched. Mature CD4 T cells in the graft are activated by allogeneic molecules and produce a cytokine storm that recruits other T cells, macrophages, and NK cells to create inflammation. B cells may be present but don’t have a significant role in GVHS.

GVHD is characterized as a bright red rash (may itch, onset may be accompanied with fever) that often begins on the face and spreads to involve the trunk and limbs including the palms and soles, profuse watery diarrhea, pneumonitis (inflammation of the lungs), and liver damage due to destruction of hepatic tissue. Bone marrow can also become the site of inflammation. For a recipient to develop GVHD, the graft must contain immunocompetent cells, the recipient must express major or minor histocompatibility molecules that are lacking in the graft donor, and the recipient must be incapable of rejecting the graft. It is acute if it occurs less than 100 days after transplant and chronic if it develops after 100 days. Chronic GVHD is more severe and difficult to treat.

Hematopoietic cell transplantation from a haploidentical donor (half matched, typically 1 or the 2 parents) carries a very high risk of GVHD. For this reason, the bone marrow from haploidentical donors is manipulated to depelete the mature T cells or purify the stem cells before attempting the transplant. Transplant to Matched Unrelated Donors (MUDs) have become the current practice, although there is a significant risk of GVHD with this type of transplant. Some centers use T cell depeltion for MUD transplants although this isn’t common practice. Transplant with unmanipulated bone marrow from MUDs is associated with a higher rate of engraftment and may provide a graft vs leukemia raction in which donor derived mature T cells may kill the rediual leukemic cells of the recipient and make relapse less likely in the case of bone marrow transplants for the treatment of leukemia (engrafted T cells recognized allogenic antigens such as HB-1 on the recipient’s hematopoetic cells and thus will attack the leukemic cells, HB-1 is a B cell lineage marker expressed by acute lymphoblastic leukemia cells and by B cells transformed by EBV).

The presence of alloreactive T cels in donor bone marrow is usually detected in lab testing by the mixed lymphocyte reaction (MLR) in which lymphocytes from the potential donor are mixed with irradiated lymphocytes from the potential recipient. If the donor lymphocytes contain alloractive T cells, these will respond by cell division. MLR doesn’t quantify alloreactive T cells. Limiting-dilution assay can more acurately count the frequency of alloreactive T cells, its too cumbersome for routine clinical use.

**Case:** healthy 7 yr old boy until he became very pale and developed petechiae (small hemorrhages) on the skin of his arms and legs, hemoglobin and platelet count was low (anemic), WBC count was low, bone marrow biospy showed very few cells and that red cell, platelet, and white cell precursors were almost completely absent, aplastic anemia (bone marrow failure, ultimately fatal) of unkown cause was diagnosed, treatment with bone marrow transplant from HLA identical brother from nucleated bone marrow cells of his brother’s iliac crests, given course of fludarabine and cyclophosphamide to eradicate his own lymphocytes, started on cyclosporin A to prevent GVHD, did well for 3 weeks after transplant and the developed a skin rash and watery diarrhea consistent with acute GVHD, patchy red rash on palms and soles, scalp and neck, no fever and wasn’t jaundiced, lungs were clear and heartbeat was normal, liver and spleen weren’t enlarged, treated with corticosteroids/tacrolimus and the rash faded but intestinal symptoms didn’t abate and diarrhea became more profuse, given rabbit antithymocyte globulin (monoclonal antibody, ATG) for 2 days which decreased the volume of hist stool and intestinal bleeding stopped, continued treated with low dose corticosteroid to keep GVHD under control

**Know that GVHD is defined as being “acute” if occurring <100 days after transplant and “chronic” if occurring >100 days.**

**Know that acute GVHD is usually easier to control with immunosuppressive drugs than is chronic GVDH.**

**Know that mature T cells among donor cells used in bone marrow transplantation or contaminating preparations of peripheral blood stem cells may recognize host tissues as foreign (a subset of T cells appear to recognize foreign MHC in spite of thymus-training against a different MHC type).**

**Know GVHD leads to a severe inflammatory disease in the recipient and, if not controlled, will lead to death.**

**Know that GVHD occurs both when MHC class I or II molecules are mismatched, and even in cases where they are matched. In these latter cases, any differences in amino acid sequences of cellular proteins in the recipient relative to the sequences of the same proteins in the donor may allow the presentation of seemingly “foreign” epitopes to the donor’s T cells. Any donor cells having receptors for these peptides will then become activated.**

**Know how the mixed lymphocyte reaction is used to detect histoincompatibility, and that the stronger the reaction, the greater the incompatibility to be expected.**

**Know that an extensive skin rash which includes the palms and soles is a typical primary sign of GVHD, and that GI tract involvement with diarrhea follows soon thereafter.**

See lymphocytes emerging from blood vessels and adhere to the basal layer of the epidermis. The basal cells begin to swell and vaculate and their nuclei become condensed as the cells die. Advanced destruction of the skin is shown by sloughing of the epidermis. Inflammatory cells also invade the crypts of the intestine and destroy normal archetecture. Cells also invade the liver and destroy hepatic ducts.

One reason that skin and GI are targeting is because the skin and intestine express a higher level of MHC molecules that other tissues. The intestinal tract is also likely to be damaged by preparative cytotoxic treatments that are given to destroy the recipient’s bone marrow. The damage induces production of cytokines, as well as inducing MHC molecules that can drive GVHD and make the tissue susceptible to immunological attack.

**Know that IFNγ production by CD4+ T cells tends to increase and prolong GVHD.**

CD4 cells in the graft that recognize foreign histocompatibility molecules become activated and produce IFNg. IFNg induces expression of MHC molecules on cells and makes GVHD worse because it provides more targets for the donor T cells.

**Know that immunosuppressive drugs used for treatment of GVHD include prednisolone, tacrolimus, and monoclonal anti-T cell antibodies such as anti-CD3 or anti-CD2.**

Treatment involves elimination of the T cells that initiate the reaction either with immunosuppressive drugs or by T cell depleting agents.

In vivo T cell depletion can also be achieved with IV injection of monoclonal antibodies such as rabbit antithymocyte globulin (ATG) and anti-CD3. This approach is also used to treat initial graft rejection in recipients of solid organ transplantation, but is associated with higher risk of lymphoproliferative disease induced by EBV. Monoclonal antibodies targeting activated, but not resting, T cells have been also used in the treatment of GVHD, and include anti-CD25 and anti-40L antibodies.

**Case 17: Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED)**

**Summary:** Millions of different T cell receptors are generated during development of thymocytes in the thymus gland. This stochastic process leads to the formation of some T cell Ag receptors that can bind to self antigens. When a T cell receptor of a developing thymocyte is ligated by Ag on thymus stromal cells, the thymocyte dies by apoptosis. This response of immature T cells to stimulation by Ag is the basis of negative selection, leading to elimination of these T cells in the thymus to abort their subsequent potentially harmful activation via apoptosis (see via TUNEL assay).

Self MHC:self peptide complexes encountered in the thymus purge the T cell repertoire of immature T cells that bear self reactive receptors. The bone marrow derived dendritics cells and macrophages of the thymus are thought to have the most signficant role in negative selection, althought thymic epithelail cells may also be involved. Myraid of self antigens, many of them organ specific, are seen in the developing thymus gland by developing thymocytes via expression of AIRE gene (autoimmune regulator). Autosomal recessive mutation in this gene leads to APECED (autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy).

APECED is also known as autoimmune polyglandular syndrome type I and is the only 1 known to have a pattern of autosomal recessive inheritance on chromosome 21. Patients have a wide range of autoantibodies not only against organ specific antigens of endocrine glands but also against antigens in the liver, skin and against blood cells such as platelets. In addition to autoimmune diseases, affected individuals have abnormalities of various ectodermal elements such as fingernails, teeth, and skin. Dysplasia (abnormal growth) in nails. Increased susceptibility with candida albicans.

AIRE was the first identified gene outside the MHC to be associated with autoimmune disease and encodes a transcriptional regulator. When Aire is knocked out in mice they developed autoimmune diseases. Extent and severity of these autoimmune disease presgressed as the mice aged. AIRE is normally epressed in the epithelial cells of the thymic medulla and weakly in peripheral lymphoid tissues. AIRE induces the expression os 200-1200 genes of the salivery galnds, zona pellucida of the ova, among others. In normal circumstances the expression of these self peripheral tissue antigens in the thymus permits negative slsection of those developing T cells that react to them. When AIRE is lacking, these antigens are not present so potentially self-reactive T cells aren’t removed from the population of T cells in the thymus and leave to cause autoimmune reactions in peripheral organs. The molecular mechanisms by which AIRE controls expression of peripheral tissue antigens in the thymic medulla are complex and involve recognition of unmethylated histone 3, transcriptional activation, and control of mRNA splicing.

Auotimmunity also accounts for the selective susceptibility to candidasis in patients with APECED because these patients have autoantibodies against IL-17A, IL-17F, and IL-22 (cytokines with a critical role in the control of fungal infections).

Primary ovarian failure has frequently been observed in females with APECED. Autoantibodies against ovarian cells are common in these patients and may cause primary ovarian failure.

**Case:** 18 mo old boy with dry skin and movements seemed sluggish, diagnosed as hypothyroidism and prescribed Synthroid (thyroid hormone), at 6 yrs old wasn’t growing at a normal rate and height and weight was below third centile for his age, X ray of his wrist showed that his bone age was 3.5 yrs, blood test showed levels of thryoid-stimulating hormones were high and indicated that he was receieving inadequate thryoid hormone replacement so the dose was increased

Had a sister that was 2 yrs older who had nail dystrophy, perioral candidiasis, hypoparathryoidism, and serum antibodies against islet cells of the pancreas (no clinically apparent diabetes), suspected inherited APECED abnormality and determined deletion of base pairs in exon 8, tested patient’s serum Ca to make sure he didn’t develop hypoparaythryoidism bt were normaly, also didn’t have anitbodies against endocrine glands, fingernails were thickened with longutidunial notches and ridging, patches of hair loss at top and back of Robert’s scalp, roots looked atophied under the microscope and was diagnosed with alopecia areta (patchy hair loss)

At 8 yrs continued to grow and TSH level was normal but continued to lose hair in patches and lost hid eyebrows, developed fissures at angles of his mouth as result of infection with C. albicans, became depressed an overdosed on Synthroid in a suicide attempt (received intensive psychotherapy)

When approached puberty his scrotum become darkly pigmented as did his areolae around his nipples, suspected that he was developing adrenal insufficiency (addison’s disease), blood test reveled high adrenocorticotropic hormone levels, perscribed steroid prednisone and Fluorinef (conserves sodium and potassium excretion)

At 18 started to bruise easily and his gums bled after brushing his teeth, low platelet count showed that he developed another autoimmune disease, idiopathic thrombocytopenic purpura

Loss of adrenal cortical hormones as result of autoimmune destruction of adrenal cortex cause his putytary gland to secrete increased ammounts of ACTH, which can be cleaved at the amino-terminal end by trypsin-like enzymes. Residual peptide is called melanocortin and stimulates melanocytes in the skin to produce melanin (brown pigment). Receptor for melanocortin also binds intact ACTH at a lower affinity. The increased amounts of ACTH and melanocrotin led to increased pigmentation of his scrotum and tissues around his nipples.

**Describe autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED = autoimmune polyglandular syndrome type 1) as a syndrome that is inherited as an autosomal recessive and has high incidence among Finns, Sardinians, and Iranian Jews.**

**Recognize that APECED 1 is associated with mutations in the gene encoding a transcriptional regulator (AIRE = autoimmune regulator), and that this gene is expressed within the thymic medulla.**

AIRE mutated mice had twice the normal number of CD4 and CD8 effector/memory cells in their lymph nodes resulting from lack of negative selection of autoreactive cells in the thymus.

**Explain that expression of AIRE appears to induce synthesis of many “organ-specific” proteins within the thymus, thus allowing their expression on antigen-presenting cells in the thymus and enabling negative selection of antigen-specific T cells.**

T cells specific for self antigens are deleted in the thymus via apoptosis.

**Recognize that autoimmune attack on the thyroid is can take several forms, with the clinical effect being due to a variety of activities. At various ends of the disease spectrum are Graves’ disease (where autoantibody to the TSH receptor mimics the activity of TSH and causes hyperthyroidism) and Hashimoto’s disease (where autoreactive cytotoxic T cells destroy the thyroid, leading to hypothyroidism). Additional cases may involve autoimmune antibodies that block TSH activity and produce hypothyroidism. Thyroid disease often has several disease-causing activities appearing at the same time.**

**When lymphocytes from Aire-deficient mice were transferred into Rag-deficient mice, which have no mature lymphocytes of their own, autoimmune disease developed in the recipient. This didn’t occur when lymphocytes from normal mice of the same strain were transferred into Rag-deficient mice. What can be deduced from this experiment? (question 3)**

Implies that Aire acts in the thymus and that the epxression of Aire in peripheral organs is less important. The lymphocytes transferred from the normal mice had left the thmus with self-reactive clones deleted, whereas this hadn’t occurred in the Aire-deficient lymphocytes. To show definitively that the thymus is responsible for the disease, the investigators transplanted either knockout or control thymuses into nude mice which don’t have a thhmus. Only the mice that receieved a KO thymus developed autoimmune disease.

Experiments with mice show that bone marrow derived cells mediate negative selection in the thymus. Bone marrow is injected from a MHC a and b mouse into an irradiated MHCa mouse, the T cells mature on thymic epithelium expressing only MHCa molecules. The chimeric mice are tolerant to skin grafts expressing MHCb molecules as long as the grafts don’t present skin-specific peptides that differ between strains anad b. Implies that the T cells whose receptors recognize self antigens presented by MHCb have been eliminated in the thymus

**Case 18: Immune Dysregulation, Polyendocrinopathy, Enteropathy X-linked Disease (IPEX)**

**Summary:** Immune system must distinguish potentially dangerous antigens from those that are harmless. Many innocuous foreign antigens are encountered every day by lungs, gut, and skin as well as seeing numerous self antigens that might bind to the spectific antigen receptors on B and T cells. Activation of the immune system by antigens such as these would be unnecssary and may lead to unwanted inflammation. Allergic and autoimmune diseases are examples of potentially destructive responses.

Unwanted immune responses are normally prevented or regulated by immunologic tolerance (nonresponsivenss of lymphocyte population to the specific Ag and arises at 2 stages of lymphocyte development. Central tolerance is the removal of self-reactive lymphocytes in central organs (case 17) and peripheral tolerance inactivates T and B cells that escape central tolerance and exit to the periphery. Defects in either of these mechanisms can lead to unwanted or excessive immune responses.

Several mechanisms of peripheral tolerance exist. One is via regulatory T cells that prevent or limit the activation of T cells, including self-reactive T cells, and the consequent destructive inflammatory process. Key cell type responsible for peripheral tolerance is the CD4 CD25 regulatory T cells also known as the natural regulatory T cells, which seems to become committed to a regulatory fate while still in the thymus and represents a small subset of circulating T cells (5-10%). CD25 (alpha chain of IL2R) is also seen on other cells, so natural T reg cells are better characterized by their expression of the TF Foxp3 which is essential for their specification and function as regulatory cells. Crucial to maintenance of peripheral tolerance. Thymic hypoplasia in humans (DiGeorge syndrome) result in impaired generation of natural Treg cells and the development of organ specific autoimmune disease. The generation of natural Treg cells in the thymus requires interaction with self peptide:MHC Class II complexes on cortical epithelial cells.

A second group of reg T cells are induces from naïve CD4 T cells in the periphery. These are CD4+ CD25- and are heterogenous including subsets known as Th3, TR1 and CD4+ CD25- Foxp3+. A rare population of CD8 T reg cells have also been identified. NK cells and NKT cells have also been shown to be able to regulate immune responses. As a group, regulatory cells respresent one mechanism in a complex system of immunologic tolerance, acting to prevent or rein in unwanted immune responses.

Breakdown in peripheral tolerance as result of a defect in Treg cells leads to allergic symptoms, GI symptoms and auotimmune disease in infancy known as Immune Dysregulation, Polyendocrinopathy, Enteropathy X linked Disease.

IPEX is a very rare disease caused by mutations in the genes for Foxp3. Foxp3 expression is restricted to a small subset of TCRa:b T cells and defines 2 pools of Treg cells (CD4 CD25high T cells and a minor population of CD4 CD25 low/neg T cells).

In humans, missense or frameshift mutations in FOXP3 result in loss of function of Treg cells and uninhibited T cells activation. The most common symptoms are intractable watery diarrhea leading to failure to thrive, dermatitis, and autoimmune diabetes developing in infancy. The dirrhea is due to widespread inflammation of the gut including the colon (colitis) resulting in villous trophy which reduces the absoptive capacity of intestinal lining and contributes to wasting. Other diseases due to immune dysregulation include autoimmune thrombocytopenia, neutropenia, anemia, hepatitis, nephritis, hyperthryoidism or hypothyroidism, and food allergies. Autoantibodies can be involved. Patients may suffer more frequent infections (sepsis, meningitis, pneumonia) although the reason is unclear. Generally have normal Ig levels except elevated IgE and their ability to make specific Ab is intact.

Ectopic expression of FoxP3 is sufficient to convert naïve murine CD4 T cells to T reg cells. Overexpression of FoxP3 in naïve human CD4 CD25- T cells in vitro will not generate potent suppressor activity, suggesting that additional factors are required. Expression and suppressor function can be induced in human CD4 CD25- FoxP3- cells by cross linking the TCRs and stimulation via the costimulatory receptor CD28 or after Ag specific stimulation. Suggests that de novo generation of Treg cells in the periphery may be a natural consequence of the human immune response.

Treg cells are anergic in vitro. They fail to secrete IL2 or proliferate in response to ligation of the TCRs and depend on IL2 generated by activated CD4 T cells to survive and exert their function. An in vitro assay that measures the ability of CD4 CD25 T cells to suppress CD4 T cells proliferation is commonly used to test Treg function (cells stimulated with immobilzed plate bound anti CD 3 and soluble anto CD28 for 3 days then assessed for proliferation by incorporation of H labedled thymidine into DNA). How Treg cells suppress immune responses is still unclear by may involve contact depende inhibition or secretion of immunnosuppressive cytokines such as IL10 and TGFb, or by directly killing their target cells in a perforin dependent way.

Other mechanisms of peripheral tolerance: T cells that are physically separated from their specific Ag (BBB) can’t be activated (immunologic ignorance). T cells that express Fas on their surface can receive signals from cells that express FasL leading to their deletion. Activation of naïve T cells can be inhibited if the cell surface protein CTLA4 binds to B7.1 on APCs. Reg T cells (CD4 CD25 Foxp3 ) can inhibit or suppress other T cells via production of inhibitory cytokines such as IL10 and TGFb.

Other mutations that can look like IPEX: IL2Ra deficiency. IL2 signaling is essential for mainteance of Treg cells. Therefore Treg activity is deficient in absence of IL2, IL2Ra (CD25) or the TF STAT5, which is important for transudcing the IL2 signal.

**Case:** Boy born at full term and developed atopic dermatitis shortly after birth (treated with skin hydration and hydrocortisone and antihistamines), at 4 mo developed intractable watery diarrhea, weight had fallen below 3rd centile, at 6 mo developed high blood glucose and gluce in the urine (type I diabetes, insulin dependent DM), diffuse eczema and sparse hair, cervical and axillary LN and spleen were enlarged, normal WBC, Hb, and platelet count, eosinophila and elevated IgE, autoantibodies detected against glutamic acid decarboxylase and pancreatic islet cells (started on insulin therapy), given parenteral nutrient to maintain weight due to persistent diarrhe and failure to thrive, dueodenal biospy showed almost total villous atrophy (absence of villa in the lining of the duodenum) with dense infiltrate of plasma cells and T cells (mononuclear cellular infiltrate), developed thrombocytopenia (platelet deficiency) and anti platelet antibodies were detected

Family history: had a brother that died in infancy with severe diarrhea and low platelet county, IPEX was suspected

FACS showed peripheral nuclear cells lacked CD4 CD25 T cells and CD4 Foxp3 positive cells, Foxp3 gene revealed a missense mutation

**Treatment:** immunnosuppresive therapy (cyclosporin and tacrolimus), bone marrow transplant from HLA matched donor (didn’t need full engraftment because only a small number of Treg cells is needed to control disease symptoms via immune regulation)

Diarrhea improved during conditioning for bone marrow transplant. Involves treatment with cytotoxic drugs that kill all rapidly dividing cells including the CD4 effector T cells responsible for the unctronolled inflammatory response in IPEX. These cells are destryed and the autoimmune response that they have produced will be dampened. Immunosuppressant drugs have the same effect inreducing the activation and proliferation of T cells and this is why they are used to treat IPEX and other autoimmune disorders.

IVIG may be used to treat IPEX because patients are unable to downregulate the immune activation triggered by infections and their disease frequently flares up on exposure to pathogens or after vaccination. IVIG is useful in forestalling infections or ameliorating their impact when they occur. Vaccination is contrainidicated in patients because of risk of disease flare up.

Use of CD4 CD25 T reg cells may have therapeutic potential in human autimmune diseases such as colitis.

**Describe the role of CD4+ CD25+ Treg cells in suppressing T cell responses to antigens and subsequently enhancing peripheral tolerance.**

**Describe IL-10 and TGF- as cytokines released by Treg cells and explain their action**

**Explain that expression of the Foxp3 transcription factor appears essential for development of Treg cells, and that failure to make Foxp3 (following a mutation in its gene) results in lack of Treg production.**

**Describe how the lack of control of T cell activation in IPEX leads to uncontrolled inflammation at various sites. Know that these activities typically include the gut, where widespread inflammation extends to encompass the colon. The subsequent production of villous atrophy results in a large drop in absorptive surface area. Clinically, this is associated with an intractable water diarrhea, failure to thrive, dermatitis, and development of autoimmune diabetes in infancy. Control of inflammation leads to improvement, at least for the short term, but bone marrow transplantation with functional Tregs is often required for permanent control.**

**Case 39: Crohn’s Disease**

**Summary:** Intestinal epithelium is important for nutrient, vitamin absorption, water handling, and secretion of waste. The gut mucosa is crucial for pathogen surveillance, mucosal barrier function, and regulation of composition of the intestinal microbial flora (microbiota). The mucosa is composed of the innermost layers of the intestinal wall adjacent to the gut lumen. And is composed of the glandular surface epithelium overlying the lamina propria, which is separated from the submucosa by a thing muscular layer (muscularis mucosae). Absorptive epithelial cells (enterocytes), and secretory goblet cells and Paneth cells are important in the gut function. Absorptive surface area is increased via invaginations that form villi. Goblet cells secrete mucus into the lumen that protects the mucosa from digestive enzymes. Paneth cells in the bases of intestinal crypts between the villi are specialized eptiheial cells that secrete enzymes and anti-microbial peptides (defensins) that prevent translocation of potentially pathogenic bacteria and toxins across the bowel wall. The submucosa is the second layer and involves autonomic nerves that control contraction of the smooth muscle to regulate peristalsis. The loos CT serosa is the outer layer of the bowel wall.

Cells of the gut mucosal innate and adaptive immune system are interspersed between cells of the mucosal epithelium and through the lamina propria and are also present as organized lymphoid organs (Peyer’s patches) and isolated lymphoid follicles. Cells and organs of the gut mucosal immune system are known as GALT and is the largest lymphoid tissue in the body. Mucosal immune system performs an important function in promoting the colonization of beneficial bacteria which contribute to nutrient digestion while mediating the destruction, clearance, and development of immunological memory to pathogens and toxins. Dysregulation of complex mechanisms respondible for mucosal immune function can result in inflammatory bowel disease (Chrohn’s and ulcerative colitis).

Neutrophils, macrophages, and DCs of the innate system express PRRs and act as sensors for potentially pathogenic bacteria in the gut. PRRs recognize lipids, carbs, Nas and peptides that are common features of pathogens. One class of these receptors are TLRs which function as innate receptors at the cell surface and in endosomal membranes. Activation by microbes triggers innate pathways that mediate inflammation and host defense. NOD proteins (NT binding oligomerization and domain containing proteins) comprise another class of PRRs that are located in the cytoplasm and bind to bacterial PGs released during infection. Both receptors activate the NFkB pathway and lead to generation of pro inflammatory response by epithelial cells including production of chemokines (CSCL8, CXCL1, CCL1 and CCL2) which attract neutrophils and macrophages and CCL20 and beta defensin which attract immature DCs in addtion to antimicrobial properties. Il1 and IL6 are also produced and activate macrophages and other components of the acute inflammatory response. The epithelial cells also express MICa and MICb and other stress related nonclassical MHC molecules which can be recognized by cells of the innate immune system.

Antigens can etner the mucosal lymphoid tissue through M cells and initiate adaptive immune responses. APCs in the gut mucosa (macrophages and DCs) take up and kill bacteria via phagocytosis and present Ags to and activate resident naïve T cells and B cells within the GALT. B cells are activated to protduce Ag specific secretory dimeric IgA antibodies which are transported into the gut lumen to neutralize pathogenic bacterial proteins required for mucosal colonization and translocation and also helps to prevent invasion by the gut’s commensal microbiota. GALT includes Th1 and Th2 cells, Tc cells, Treg cells, and T17 cells in addition to B cells. T17 cells may have a protective role in preventing gut inflammation and maintaing homeostasis within the bacterial flora. In absence of infection, DCs present antigens to naïve T cells in the mucosa and tend to stimulate production of Treg cells and avoid a damaging inflammatory response to commensal microbiota.

Crohn’s disease is a disorder of the mucosal immune system characterized by inflammatory lesions that can involve the entire GI tract from mouth to anus. 40-50% of pediatric cases involve the ileum and colon (ulcerative colitis typically involves only the colon and rectum). Biopsy evidence of inflammatory cell infiltrate is transmural in Crohn’s in that it involved the epithelium, LP, and adventitial layers of the bowel wall. As a result, fistulas and bowel abscesses are frequent complications. The presence of extra intestinal inflammatory manifestations of IBDs invluding the skin (erythema nodosum, pyoderma gangrenosum), joints (arthritis), and eyes (uvetitis) shows that IBDs are systemic inflammatory diseases. GI cancers occur with increased frequency in patients with IBDs indicating the role of chornic inflammation in increased risk for malignancy. See small intestinal narrowing in barium study of Crohn’s patients.

Dysreglation of mucosal immunity to get commensal microbiota and a resulting impaired mucosal barrier function. Found that NEMO deficiency or in Treg cell deficiency develop IBD shows that innate immune system and Treg cells are essential in maintaining intestinal homeostasis. Disruption in genes for IL2 (Treg function) or IL10 (immunosuppressive cytokine) are protected from the development intestinal inflammation when raised in germ free conditions which shows a role for host-microbiotia interactions in initiating IBD pathogenesis. IL23R isoderms show susceptibility and protection indicating the complex roles of Treg and T17 cells in intestinal inflammation, Il23 is required for T17 maintainece and survival. T17 and Treg cells have reciprocal relationships because both compete for cytokine TGFb for their induction, and T17 cells but not Treg cells also required T23R.

Crohn’s patients have been identified with mutations in NOD2 intracellular innate receptor expressed in macrophages and epithelial cells that binds to muramyl dipeptides from bacterial cell walls and activates the production of inflammatory cytokines. Have impaired secretion of defensins when exposed to invasive bacteria (Blau syndrome, AD with granulomatous inflammation of eyes, skin, and joints also due to NOD2 mutations).

Autophagy (cell stress response pathway) delivers intracellular organelles and cytoplasmic contents to the lysosomes for degradation and is important for alling off and eliminating bacteria that escape into the cytosol or enter it directly. ATG16Li and IRGM genes implicate defects in autophagy in Crohn’s possibly because inefficient elimination of microbes from the cytosol leads to sustained cytokine secretion and Paneth cell dysfunction, resulting in intetinal inflammation. Cell stress caused by misfolded proteins, metabolic factors and microbes activate the unfolded protein response which activates TF XBP1 which regulates genes important for proper function of the immune system. Defects in XBP1 can be found in Crohn’s patients and suggest that abnormalities in cell stress response may result in intestinal inflammation and decreased survival and Paneth cells.

**Case:** previously healthy 8 yr old developed painful swelling and redness and warmth of his toe, mouth elcers (aphthous ulcers), no fracture or injury, over next 2 months developed frequent poorly localized abdominal pain. Passing stools was difficult and had difficulties with constipation, no bloody stool or sensation of incomplete bowel evacuation, severity of ab pain, toe swelling, and oral ulcers seems to wax and wane over the next 2 months, daily low grade fevers, abdominal pain more severe and began to pass stool more frequently, faitgue and listless, poor appetite, lost weight, painful raised red lesions on right shin (acute erythema nodosum), pain in jaw, looked tired and pale, ab exam sowed no focal tenderness or masses, inflammed anal skin tags found and rectal exam showed no tenderness, dissures or occult blood, WBC count elevated, platelets elevated, ESR and CRP elevated, endoscopy found ulceration of the esophagus and SI and a perianal fistula, neutrophilic infiltration of the esophageal and ileal lesions, active colitis with rare crypt abscesses but no granuloatous inflammation, developed abdominal mass in RLQ and ab ultrasound and CT revealed large abscess of ileum and cecum (distal SI, ileum and cecum were resected and multiple smaller lesions were drained)

No family history of inflammatory bowel disease or autoimmune illness

Platelets were elevated because the are acute phase reactant and are elevated during systemic inflammatory responses (cytokines lead to increased platelet release and production in the bone marrow)

**Treatment:** corticosteroids, 6-mercaptopurine (meabolized to 6 thioguanine which inhibits synthesis of purines needed for DNA and RNA synthesis, toxic to rapidly deviding cells that required NA synthesis, lymphocytes mediating autoimmune and inflammatory responses are rapidly dividing and can be targeted by antimetabolite drugs), infilximab and adalimumab (inhibit TNFa, infilimab is chimeric mouse-human and adalimumab is fully humanized, some patients treated with infilximab generate neutralizing antibodies against the mouse portion of the chimeric mab so it isn’t effective, because adalimumab is fully humanized the problem of neutralizing antibodies is diminished), natalizumab (mab against cell adhesion alpha 4 integrin, used by lymphocytes to bind to VCAM1 on endothelial cells to allow them to migrate from the vasculature to sites of inflammation to which they are attracted by chemokines, inhibiting T cell homing to the gut reduces extend of inflammation and decreases disease symptoms)

Explain that role of mucosal innate immunity in Crohn’s disease.

Describe the pathogenesis of Crohn’s disease.

Explain how to differentiate between Crohn’s disease (regional enteritis, [abdominal pain](http://en.wikipedia.org/wiki/Abdominal_pain), [diarrhea](http://en.wikipedia.org/wiki/Diarrhea), [vomiting](http://en.wikipedia.org/wiki/Vomiting), [weight loss](http://en.wikipedia.org/wiki/Weight_loss), skin rashes, arthritis, eye, NOD2 gene)and Ulcerative colitis ([colitis](http://en.wikipedia.org/wiki/Colitis), [colon](http://en.wikipedia.org/wiki/Colon_(anatomy)), [ulcers](http://en.wikipedia.org/wiki/Peptic_ulcer), crypt abscesses, [diarrhea](http://en.wikipedia.org/wiki/Diarrhea) mixed with blood).

Crohn’s biopsy: discrete granuloma composed of macrophages, giant cells and epitheloid cells, marked infiltration of lymphoid cells, plasma cells, no necrosis

Ulcerative colitits: crypt abscess composed of transmigrated neutrophils and surrounding epithelium exhibits features of acute mucsoal injury.

**Case 43: Pemphigus vulgaris**

**Symptoms:** Due to disorder in adaptive immune response directed against self antigens. Type II autoimmune disease. Autoantibodies that interact with structural protein of the epidermal cells of the skin resulting in skin cells coming apart from each other leading to skin blistering and destruction. Autoantibodies against desmoglein-3 which is a protein comopnent of the desmosome (intercellular junctions that link skin cells and other epithelial cells tightly to each other). Members of the cadherin family that effect interceullar adhesion in a calcium dependent manner. Disruption of desmoglein causes blisters to form in the skin and on MM. Extensive sloughing of the skin may ensue. Lesions can coalesce and produce a large plaqu that has a most durface that is oozing and crusts of dried serum from blister fluid. Infants borne to mothers with the disease have transient period of skin blistering during the neonatal period. See epidermal suprabasal vesicles. See deposits of IgG in the intercellular spaces of the entire stratum malpighii of the epidermis.

Autoantibody is of the IgG4 subclass and doesn’t fix complement. Binding of the autoanitbody to the antigen causes upregulation of serine proteinase activity on the surface of the epidermal cells and results in the proteolytic digestion of desmoglein-3. Response evolves to involve epitopes other than the one against which antibodies are initially directed. Antibodies present before onset of disease are specific for epotopes in the extracellular part of the molecule nearest the cell membrane. These antibodies don’t bind to cell surface desmoglein or transfer disease when injected into mice. With disease onset, the antibodies specific for the domains farthest away from the cell surface become detectable and bind cell surface desmoglein and when ingected into mice they induce the disease. The process by which immune response initially targets epitopes in one part of an antigenic molecule and thenprogresses to other non cross reactive epitopes of the same molecule is called intramolecular epitope spreading and correlate with the clinical progression of disease. In pemphigus it is only with epitope spreading that disease causing antibodies are made.

Isotype switching is required to make IgG4 and is stimulated by IL4 released by Th2 cells. May find that patients T cells make more IL4 and undergo more isotype switching than those of asymptomatic people.

Encountered most frequently in Ashkenazi Jews and has a strong association with HLA-DR4 and HLA-DQ3. Most are heterozygous for this haplotype. Unaffected relatives of patients who bear the same HLA frequently have antibodies against desmoglein-3 but they are IgG1 and don’t cause disease (react to different epitope, bind complement). In other ethnic groups the disease is usually associated with HLA-DR14 and HLA-DQ5. Association with HLA-DR4 is due to the way that the MHC2 can bind peptides. The actual peptides that can be bound by any given MHC molecule are determined by the particular pattern of amino acid binding sites in the peptide binding cleft of the MHC via H bonding. The beta chain of MHC2 molecule CR is designed as DRB1. 22 known subtypes of this chain have been identified amoung those with DR4. DRB1\*0402 is associated with PV. It differs in the amino acid at position 71 which lies in one of the binding pockets in the peptide binding groove of the MHC. It is a negatively charged glutamic acid whereas all the other varients have positively charged lysine or arginine. Only DRB1\*0402 can bind antigenic peptide derived from desmoglein-3 and present it to T lymphocytes.

Was frequently fatal in the past before control with immunosuppressive drugs because the epidermis is an important barrier to the entry of pathogens into the body. Extensive blistering and sloughing of the skin destroyed the barrier and often resulted in bloodstreem septicemia with s. aureus.

**Case:** 55 yr old Ashkenazi Jew, persistant irritation in his throat, became very hoarse, sore developed on his right check when he was very stressed, 2 weeks later had numerous erosions and ulcers in mucosa of mouth and gums, plalate, ucula, tongue, scalp, and neck, biopsy showed disruption of epidermal layer of skin, IF showed depositis of IgG in an intercellular pattern in the epidermis, antibdoy against desmoglein-3 (pemphigus antibody), after steroid treatment developed persistent cough, fever, chest pain (intestitial penumonia due to penumocystis jirovecii), cyclophosamide because his condition wasn’t responding to steroids, rinsed mouth daily with hydrogen peroxide and decadron corticosteroid, new lesions are injected with corticosteroid

Pneumocytstic gjirovecii is an opportunistic pathogen that is a frequent cause of pneumonia in immunosuppres patients. High dose corticosteroids suppressed T cell functions and trafficking and caused increased susceptibility to this pathogen).

**Treatment:** prednisone, cyclophosphamide (alkylating agent that interferes with DNA synthesis and stops cell division in lymphocytes to suppress immune reactions, side effects include anemia, thrombocytopenis and hair loss), monomeric IgG in IVIG binds to high affinity Fc receptors on macrophages and causes release of immunosuppresive cytokines such as TGFb, IL10 and IL1R antagonist

**Describe how desmoglein 3 is the target for the autoimmune response leading to pemphigus vulgaris, and that it is a protein component of the desmosome that acts as an intercellular connector between skin cells.**

**Explain that there are several variants of the HLA-DR4  chain, but in pemphigus vulgaris, the variant that is strongly linked to the disease has a differently charged amino acid residue (relative to the charge found in the other HLA-DR4 variants) in its peptide-binding groove.**

**Explain that the specific peptide from desmoglein-3 that has been associated with induction of the autoimmune response only binds to the variant HLA-DR4 found in pemphigus (e.g. it is the immunodominant epitope for this haplotype) and not to the other forms of HLA-DR4.**

**Describe that glucocorticoids and cytotoxic drugs, such as cyclophosphamide, are used in treatment of this disease.**

**Case 44: Celiac Disease**

**Summary:** Also known as gluten-sensitive enteropathy. Immune mediated inflammatory disease of the GI tract caused by a permanent sensitivity to gluten (mixture of proteins prsent in the grains of wheat, barely, and rye). Gluten is degraded into antigenic peptides that trigger and immune process in the gut leading to diarrhea, malabsorption and ultimately nutrieitional deficiencies and failure to thrive.

Perhaps the most common genetic disorder (0.5-1% of the population). Principal genetic determinants have been identified as the highly variable HLA II DQA and DQB genes located on chromosomes 6 in the MHC. Prevalence varies by population. Variable presentation and delay in onsetset of symptoms probably leads to it beging underdiagnosed. 90-95% of patients express HLADQ2 and the remainder express DQ8. Clusters in families. First degree relative of affected patients have a 2-5% prevalence of symptomatic celiac disease and up to 10% may have histological evidence of the disease even in absence of symptoms. Patients with type 1 DM, Down’s, Turn syndrome, Williams syndrome (congenital facial and cardiac abnormalities with hypercalcemia), autoimmune thryoiditis, or selective IgA deficiency are at higher risk thn the rest of the population of developing celiac disease.

Children usually present between 6-24 months with abdominal distension, diarrhea, malabsorption, and weight loss. Anemia, irritability, and muscular wasting are often observed. Rarely, kids in celiac crisis are seen with severe diarrhea, dehydration, and marked disturbance in electrolyte balance (need immediate IV corticosteroid treatment). Older children may have more subtle symptoms but will also have diarrhea, nasusea, vomiting, and abdominal pain. Celiacl disease with constipation is seen in some patients. Atypical features include hypoplasia of the enamel of the permanent teeth, osteopenia/osteoporosis, short stature, and delayed puberty. Dermatitis herpetiformis (intensely itchy rash consisting of small solid papules and water filled vesicles) is a dermatologic manifestation of the disease. Small bowl biopsies in patients with derm herpetiformis reveal lesions that are consitent with gluten sensitive enteropathy. Both the rash and GI lesions remit if gluten is removed from the diet.

Adult can also develop the disease. Iron deficient anemia that doesn’t respond to oral therapy is the msot common presentation. GI symptoms such as dirrhea and flatulence sometimes predominate. Also important to consider in patients with unexplained bone fractures, transminitis, or neurologic symptoms (peripheral neuropathy or ataxia) and in women with infertility.

The exogenous Ag is gluten (mixture of gliadin and glutenin polypepetides present in wheat). Ingested proteins are usually degraded into small reidues by gastric, pancreatic, and intestinal enzymes during transit through the GI tract. In most cases, the residues aren’t appropriate stimulators of the immune response because they aren’t big enough to bind to HLA molecules. However, gluten contains a proline-rich peptide of 33aa that survives transit through the GI tract and arrives intact in the small bowel. Peptide passes through the GI lining into the subeptihelial space. This trafficking is usualy prevented by competent tight intercellular junctions of the GI epithelium. Factors may damage the epithelial barrier (infection, gluten induced upregulation of zonulin intestinal peptide that regulates tight junctions, or mechanical stress).

In the subepithelial space, the peptides are modified by enzymes including TTG that increase their antigenicity. The Ag are then picked up and presented by APCs to CD4 T cells to initiate an inflammatory response that is self perpetuating in the presence of gluten and damages the GI tract. The TTG enzyme deamidates the peptide and makes it more antigenic and has also been ientified as an autoantigen. The key genetic determinants are HLA-DQ2 or HLA-DQ8.

After presentation of the peptides to CD4 cells, and inflammatory response is initiated that is characterized by CD4 Th1 response and production of IFNg as the predominant cytokine. It is likely that some of the intestinal damage is mediated via a bystander effect of IFNg but other mechanisms may be involved. Exam of GI lesions in patients show infiltration of the intestinal mucoda by bother CD4 lamina propria lymphocytes and intraepithelial CD8 T cells.

Anti-TTG Ab are of clinical importance in diagnosis and in tracking disease activity. It is proposed that hapten-carrier-lke complexes form between TTG and complexes of gliadin, which activate helper T cells that then provide help to TTG-specific B cells. When gluten is removed from the diet, Ab levels decrease and the inflammation/injury resolve.

The innate immune system may also be stimulated by gluten. Gluten dervied peptides induce the release of IL15 by intestinal eptielial cells which activates DCs in the lamina propria and leads to upregulation of cell surface MICa expression by epithelial cells. Potentially cytotoxic CD8 intraepithelial lymphocytes in the mucosal epithelial can be activated via the NKG2D receptors, which recognize MIC-A and then kill MIC-A expressing epithelial cells. These innate response may create some intestinal damage and may also induce the costimlatory moelcules necessary for initiating an Ag specific CD T cell response to other parts of the alpha gliadin molecule. The ability of gluten tomstimulate both innate and adaptive immunity may explain its unique ability to induce CD.

Highly sensitive and specific serologic tests are used in diagnosis. Also important to monitor compliance with a gluten-free diet because no anti-gluten Ab can be detected when patients avoid gluten completely. Initial screening tests for IgA Ab against human recombinant TTG (tissue transglutaminase). Testing for anti endomysium IgA is frequently used but measurement of this test is observed dependent and is therefore subject to error. Positive titers of anti-TTG and anti-endomysium Ab are almost 100% successful in predicting celiac disease lesions in sympomatic patients. Anti-gliadin Ab are less predictive because elevated levels of anti-gliadin IgG are found in patients with inflammatory bowel disease and in healthy people. IgA deficiency occurs in 2% of kids with CD and can lead to misinterpretation of low Ab levels so its important to measure IgA levels also. Patients with known IgA deficiency should be screened for anti TTG IgG Ab. All suspected cases should be confirmed by a SI biopsy. Characteristic pathology includes partial to total villous atrophy and lymphcoyte infiltration with crypt hyperplasia.

Testing is recommended in asymptomatic kid who are in high risk groups (type 1 DM, autoimmune throiditis, down’s, turner, williams, IgA deficiency, and first degree relatives of CD patients) starting at age 3 as long as they haven’t been put on a gluten free diet. Reasoning behind testing asympotmatic kids is that CD can be clinically difficult to detect, but there is reason to believe that ongoing inflammation in the gut has deleterious effects, so it should be diagnosed and treated as early as possible. An initial negative screen doesn’t preclude its later emergence in some people. Repeat screening for Type 1 diabetes and patients with down’s and first degree relatives with CD should be performed periodically. An alternative to interval testing is to HLA type the high risk individuals. In absence of HLA DQ2 or DQ8, CD is very rare.

Untreated disease leads to signficant illness and increased risk of mortality. Untreated kids are most likely to have problems with growth and decreased bone mineralization which will be corrected with a gluten free diet. Untreated women are more likely to have spontaneous abortions, babies with low birth weight, and shorter duration of breastfeeding. There is also an increased in mortality due to cancer in adults with celiac disease, primarily enteropathy-associated T cell lymphoma. Strict compliance with gluten-free diet may reduce the risk of cancer compared with those patients who don’t comply.

**Case:** 12 mo old girl, became irritable and stopped gaining weight, distended abdomen, large foul-smelling stools and lost weight, becoming progressively weaker, grew normally until 15 mo, appeared pale and chronically ill, low percentaile for weight, height, and head circumferance, mildly dehydrated, significant muscle wasting in butt, legs, and arms, anemic (low Hb), mild elevation of liver enzymes (transminitis, aspartate aminotransferase, alanine aminotransferase), positive for anti-endomysium IgA (Ab against CT that sheathes m, endomysium), anti tissue transglutaminase (TTG) IgA, anti gliadin IgA and IgG, upper GI endoscopy revealed edema and flattening of the mucosal folds in the duodenal bulb, biopsies from stomach and deuodenum showed subtotal to total villous atrophy and increased intraepithelial lymphocytes in the second portion of the duodenum and duodenal bulb

Family history: parents of Irish American ancestry were healthy as well as her 3 yr old sister, no history of disease in children of the family, no history of autoimmune diseases including type 1 DM

**Treatment:** gluten free diet, nutrtional counseling

Potential therapy: bacterial propyl endopeptidase could potentically catalyze the brakdown of the 33-mer peptide and prevent it from interacting with TTG. Removing the 33-mer and its antigenic epitopes from the subeptiehlial space would eliminate the interaction of HLA DQ2 bound peptides with T cells and halt the inflammatory process.

**List the main synonyms for celiac disease (celiac sprue and gluten-sensitive enteropathy).**

**Describe the nature of gluten.**

**Describe that the classic time for disease expression in infants is 6-24 months, and is initially associated with weight loss and the appearance of being malnourished.**

**Contrast histological features of the duodenal mucosa from celiac disease and normal mucosa (Fig 36.2).**

Flattened mucosal surface, crypt hyperplasia, extensive inflammatory infiltrate. IF staining of mucosa of SI shows close association of TTG, APC (stained via HLA-D), and CD3 T cells.

**Explain that avoidance of gluten in the diet allows normal growth and development.**

**Describe the use of measuring anti-gliadin IgA, anti-gliadin IgG, and anti-TTG (tissue transglutaminase) in screening for celiac disease.**

**For celiac disease etiology, describe how a gliadin-derived, proline-rich 33-aa peptide is modified in the subepithelial spaces in the small bowel, such that it is capable of binding directly to certain MHC II molecules (Fig 36.3). Explain that restriction to certain MHC II molecules leads to the disease clustering in families expressing those haplotypes.**

Inflammation of the Si is caused by CD4 T cells responding to peptides dervied from gluten that are deamidated by TTG and presented by HLA-DQ8 and HLA-DQ2.

**Explain why oatmeal is not safe, even though oats do not contain gluten.**

Can be cross contaminated with wheat as result of storage in silos that hold both types of grains. Patients newly diagnosed with CD shouldn’t eat oats, but it can be reintroduced in small amounts once the disease is in remission.

A level of 220 p.p.m. gluten has been arbitrarily designed as gluten free but there is evidence that even very small amounts of gluten are toxic to CD patients. And a new limit of 20 p.p.m. is beging considered.